TACKLE

Study Type: 21-Day Dermal - Rabbits.

Accession Number: 071311.

MRID Number: Not assigned.

Sponsor: Rhone-Poulenc Inc., Monmouth, NJ.

Contracting Laboratory: Food and Drug Research Laboratories, Inc.

Testing Facility: Food and Drug Research Laboratories, Inc. (Lab No. 6718).

Date: February 5, 1981.

Material: Tackle 2S
Purity 21.1 percent

PROTOCOL

Procedure: New Zealand white rabbits, age unspecified, supplied by H.A.R.E., Hewitt, New Jersey, weighing approximately 2.0-4.0 kg, were housed individually in hanging wire mesh cages in a temperature-, humidity-, and light-controlled environment. The animals were given commerical rabbit feed (Charles River Rabbit Chow) and tapwater ad libitum. The animals were acclimated for 10 days prior to the study initiation and were randomly assigned to the study groups.

The back of each animal was clipped free of hair prior to the initiation of the study and weekly thereafter to keep it relatively free of hair. From each test group (10 animals/sex/dose), 5 males and 5 females were further prepared by abrading the test site (sites were abraded weekly thereafter) using a clean hypodermic needle to penetrate the stratum corneum but not cause bleeding.

The test material was mixed in aqueous NaOH and adjusted to a pH of 7.5-7.6 to obtain a solution that was to be applied so as to achieve dose levels of 0, 100, 300, and 1000 mg/kg/day. The control group received

the NaOH solution only. The dose volume for each dose level was as follows:

Table 1. Dose Volume (ml/2.6 kg of Body Weight)

Group (mg/kg/day)	Application Number		
	1-3	4	5-15
0	10	10	5[a]
100	1	1	1
300	3	3	3[b]
1000	10	5[a]	5[c]

[a] Due to overt toxicity in the 1000 mg/kg/day group, the control and high dose volumes were reduced starting on days 4 and 5 of the study, respectively.

[b] Analysis of the dose solutions found these applications equivalent to 342 mg/kg/day.

[c] Analysis of the dose solutions found these applications equivalent to 570 mg/kg/day.

The animals were exposed to test material for a 6-hour period, 5 consecutive days, for 3 weeks. The treated skin was covered with two layers of gauze and an occlusive binder. The wrappings were removed at the end of each daily exposure and the treated areas were wiped with clean gauze.

Clinical Observations: The animals were observed daily for signs of toxicity and twice daily for mortality.

Body Weights and Food Consumption: Individual body weights and food consumption were recorded twice weekly.

Dermal Irritation: The animals were observed for dermal irritation daily, and the observations were quantified using the Draize (1975) method of dermal irritation scoring.

Blood and urine samples were taken from 5 animals randomly selected from each sex and group before treatment and at termination of the study. Animals were fasted 14-16 hours prior to blood drawing. Urine samples were collected during 14-16-hour fasting period.

Hematology: Hematological determinations included total and differential leukocyte counts, erythrocyte count hematocrit, hemoglobin, and platelet count.

Clinical Chemistry: Serum or plasma was assayed for alkaline phosphatase (AP), urea nitrogen, glutamic pyruvate transaminase (GPT), glutamic oxaloacetic transaminase, calcium, potassium, lactic dehydrogenase, glucose, bilirubin (total and direct), total cholesterol, albumin, globulin and total protein.

Urinalysis: Urinalysis included appearance, specific gravity, blood, protein, pH, bilirubin, urobilinogen, ketones, glucose, microscopic examination of sediment (e.g., crystals, bacteria, cells).

Pathology: Tissues and organs of surviving and moribund animals were examined for gross and histopathological changes.

Organ Weights: Brain, heart, kidneys, liver, adrenals, pituitary, thyroid and parathyroid, ovaries, and testes were taken from all animals and weighed.

Tissues Preserved and Examined: Representative sections of the following tissues were examined: untreated skin, treated skin, kidneys, liver, and "grossly abnormal tissue."

Statistics: Analysis of body weight, food consumption, clinical chemistry, and organ weight data were performed using a one-way analysis of variance. Differences between groups were identified using the least significant difference test. Dermal irritation and urinalysis data were analyzed using the Mantel-Haenszel chi-square test. Pathology incidence data were analyzed using a chi-square test with Yates correction for 2x2 contingency tables. Results were considered significant when $p<0.05$.

RESULTS

Mortality: Nineteen of twenty high dose (1000 mg/kg/day) animals died or were sacrificed moribund during the study. The distribution of the deaths observed throughout the study are summarized in the following table:

Table 2. Summary of Mortality

Group[a] (mg/kg/day)	Days on Study							Total Mortality
	3	4	5	6-7	8	9-14	15-21	
0	-	-	-	-	-	-	1M, 1F	1M, 1F
100	-	-	-	-	1M, 0F	-	-	1M, 0F
300	-	-	-	-	-	1M, 0F	1M, 0F	2M, 0F
1000	3M, 0F	1M, 4F	4M, 4F	1M, 1F	0M, 1F	-	-	9M, 10F

[a]Control Group: 11 males, 9 females; other groups: 10/animals/sex.

Daily Observations: Mortality seen in the high dose group were preceded by the following signs of toxicity: respiratory difficulty which was seen in 4 males and 3 females, nasal discharge (3 males and 3 females), excessive salivation (6 males and 3 females), ataxia (2 males and 2 females), and tremors (2 males). Animals in the other groups, including control, displayed intermittent "diarrhea, bloated appearance and decreased activity," but with no consistency or pattern.

Dermal Irritation: As stated by the authors, "At all dose levels, repetitive daily application of the test article caused severe dermal irritation with eschar formation." It was not specified if the irritations observed were of equal severity at the abraded and unabraded skin sites. Average daily Draize scores were as follows:

Table 3. Mean Skin Irritation Scores - Males

Group (mg/kg/day)	Erythema/Eschar				Edema			
Day	0	100	300	1000	0	100	300	1000
1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1
2	0.0	0.5	0.6	0.7	0.0	0.4	0.2	0.2
3	0.0	0.8	1.0	1.0	0.0	0.5	0.6	0.7
4	0.0	1.3	1.6	1.4	0.0	0.6	0.9	1.1
5	0.0	2.2	2.3	2.8	0.0	1.4	1.6	2.3
6-7	0.0	3.1	3.9	-	0.0	2.5	3.0	-
8	0.0	3.7	3.7	-	0.0	2.5	2.8	-
9-14	0.0	3.8	3.9	-	0.0	2.8	3.1	-
15-21	0.1	3.1	3.9	-	0.0	2.4	3.0	-

Table 4. Mean Skin Irritation Scores - Females

Group (mg/kg/day)	Erythema/Eschar				Edema			
Day	0	100	300	1000	0	100	300	1000
1	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0
2	0.4	0.3	0.5	0.6	0.0	0.3	0.0	0.0
3	0.3	0.8	1.0	0.8	0.0	0.3	0.4	0.5
4	0.3	0.8	1.0	1.8	0.0	0.7	1.0	1.1
5	0.7	1.8	2.7	4.0	0.3	1.2	2.3	2.0
6-7	0.6	3.2	3.9	-	0.4	2.3	2.9	-
8	0.2	3.4	4.0	-	0.2	2.4	3.0	-
9-14	0.2	3.3	4.0	-	0.2	2.3	3.1	-
15-21	0.4	2.9	4.0	-	0.3	2.3	3.0	-

Body Weights and Food Consumption: Mean body weights and food consumption were similar to control for the low and mid-dose animals throughout the study.

Body weights were 9 percent and 13 percent lower than controls for high dose males and females, respectively, at day 4 of the study. Further

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comparison could not be made due to the deaths of high dose animals prior to day 7 of the study. Food consumption was 50 percent lower for these animals when compared to controls.

Hematology: Although "no significant differences were observed between any groups in any of the parameters analyzed" comparisons could not be made for the high dose animals due to the high mortality rate prior to day 7.

Clinical Chemistry: Scattered changes occurred, but were not of biological significance. Comparisons could not be made for the high dose animals due to the high mortality rate prior to day 7.

Urinalysis: Although "no significant differences were observed between groups in any parameter during the study" Comparisons could not be made for the high dose animals due to the high mortality rate prior to day 7.

Gross Pathology: Gross pathologic examination of the high-dose animals that died or were sacrificed in extremis during the study did not identify a treatment related pattern of pathologic changes.

In the animals that were sacrificed at the end of the study, the most frequent findings were white streaks or spots in the liver and accumulation of mucus or mucus-like material in the intestinal tract. The white streaks or spots on the liver, which occurred in 2-8 of the animals per sex in all groups, were attributed to generalized infection or active or healed lesions of a coccidiosis infection that was confirmed histologically. The mucoid accumulations, found in 1-3 animals per sex per group, were said to be due to subacute inflammation or to parasitic organisms.

Distinct differences between the control and dosed animals were noted in the appearance of the treated areas of skin. Animals receiving the test material had higher incidences of hemorrhage, discoloration, crust formation, and other changes (Table 5). No differentiation was made between the abraded and nonabraded sites in the treated animals.

Organ Weights: Organ weight data were determined at necropsy for the control, low-, and mid-dose animals; data were not presented on the high-dose animals that died during the study. With one exception, no statistical differences were found between the control and treated animals in the absolute organ weights or the organ weights relative to body or brain weight. The female animals of the mid-dose group had adrenal gland weights relative to body weight that were 35 percent greater than the control ratio, a statistically significant difference. The mean weight of the adrenal and the adrenal-to-brain-weight ratio were not statistically different for this group. It was also noted that these animals had slightly lower terminal body weights than the control and low-dose animals which may have contributed to the apparent increase in relative adrenal weight.

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Histopathology:

Skin: Histopathologic examinations were conducted on sections of treated and untreated skin, liver, kidneys, and any grossly abnormal tissues from all study animals. Only sections of treated skin were observed to have lesions that differed in severity and incidence between the control and treated groups. Animals in all of the groups administered the test article had focal or diffuse eschar formation with acute inflammation and necrosis of the epidermis and outer dermis. In addition, the study's pathologist stated that "Scattered epidermal ulcers (active, healing, or healed) with overlying crusts or eschars and underlying acute inflammation, which at times extended to the subcutaneous muscle, were also frequently seen in these animals." High-dose group animals tended to have a higher incidence of eschar formation than the other groups, but less of an incidence of epidermal thickening (hyperplasia). In the low- and mid-dose animals, acute or chronic inflammation, fibrosis of the superficial dermis, and regeneration of the injured skin were prevalent. None of the incidences of these changes appeared to be related to dose (Table 5). In the control group, a low incidence of focal acute or chronic inflammation, crust formation, and epidural thickening occurred, most likely a result of shaving and vehicle administration. No differentiation was made between abraded and unabraded skin sites.

Liver: In all groups, 1-6 animals per sex were found to have single or multiple cellular necrosis and inflammations in liver tissue, and 4 animals had lesions of the liver resulting form coccidiosis infection.

Kidney: In kidney tissue, 2-3 animals per sex per group in all groups showed focal areas of nonsuppurative chronic interstitial nephritis with tubular dilation and interstitial fibrosis.

Intestine: Several animals (9 out of a total of 80) had inflammation, necrosis, and/or coccidiosis of the intestine, all of which were considered characteristic of mucoid enteropathy. These lesions were felt to have resulted from disease processes common to laboratory rabbits and not a result of treatment.

Histopathologic examination of the high-dose animals that died during the first week of the study did not reveal any common pattern of pathology. These deaths were attributed to an acute toxic effect of the test material. Histopathologic examination was conducted on adrenal tissue of 5 males and 2 females from various groups; mid-dose females were not examined for a possible lesion related to the observed increase in adrenal weight relative to body weight. One male animal of the mid-dose group was found to have necrosis of the adrenal gland.

Table 5. Summary of the Incidence of Gross Pathologic and Histopathologic Changes in Skin

Observation	Group (mg/kg/day) and Animals/Sex/Group							
	0		100		300		1000	
	M	F	M	F	M	F	M	F
	11	9	10	10	10	10	10	10
Gross								
Diffuse or focal discoloration; hemorrhage	3	0	1	4	5	2	10	10
Sores	1	3	5	3	4	9	1	0
Crust formation	0	0	6	7	7	8	1	0
Focal scars, dryness, scales, or other[a]	1	1	12	10	13	10	9	4
Histopathologic								
Eschar formation, necrosis, ulceration	0	0	5	9	7	9	9	10
Epidermal thickening (acanthosis), hyperkeratosis	3	4	7	9	9	10	1	0
Inflammation, acute	4	7	9	10	8	10	10	10
Inflammation, chronic; fibrosis	2	4	7	8	10	9	6	5

[a]More than one observation per animal is possible and therefore the total is greater than the number of animals examined.

CONCLUSIONS

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Conclusions: Daily application of 1000 mg/kg/day resulted in 19/20 animals dying by day 8 of the study and thereby the termination of this high-dose group. The necropsy and histopathologic examination of the organs of these high dose animals did not reveal any pattern of pathology.

No systemic effects were reported for the hematology, clinical chemistry, and urinalysis parameters examined in this study for any of the groups that survived the study (evaluations could not be made for the high dose group [1000 mg/kg/day] due to the high mortality rate by day 8 of the study). Pathology was unremarkable for these groups as well.

Dermal irritation was observed at all treatment groups (100, 300, and 1000 mg/kg/day) beginning on day 2 and continuing throughout the study.

The following LELs and NOELs were identified in this study:

Systemic NOEL = 300 mg/kg/day.
Systemic LEL = 1000 mg/kg/day (19/20 animals died by day 8).
Skin Irritation NOEL: Not established.
Skin Irritation LEL: 100 mg/kg/day (LDT).

Classification: Supplementary. (Loss of 19/20 of the high-dose animals by day 8 resulted in the study effectively having only two dose groups and prevented meaningful evaluation of possible treatment related effects. In addition, histopathologic examinations were limited to the liver and kidneys in all animals and to organs with gross lesions; consequently, toxic effects on tissue and organ structure in animals of the low- and mid-dose groups may have been overlooked).

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